

## **POST-TRANSLATIONAL STRATEGIES FOR ENHANCING BIOSYNTHETIC PATHWAY EXPRESSION AND ACTIVITY.**

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The past decade has seen considerable advancements in synthetic biology tools for controlling protein and biosynthetic pathway expression. For example, low cost DNA synthesis and the standardization of genetic parts has enabled high through pathway refactoring, and riboswitches and ribosome binding site engineering has enabled the fine tuning of translational rates. As a posttranslation strategy, synthetic protein scaffolds that co-localize pathway enzymes and enhance chemical biosynthesis have also been demonstrated. In this work, we demonstrate that controlled intracellular trafficking of membrane-associated enzymes can also be used to tune pathway expression and enhance activity. Using yeast ester biosynthesis as a model system, we demonstrate two separate approaches to post-translational control over pathway expression and function. In the first, we have engineered mitochondrial and lipid droplet targeting in an alcohol-O-acyltransferase, Eeb1. Eeb1 natively localizes to mitochondria, expression levels are low, and increased transcript levels do not produce corresponding increases in expression and function. Positive correlations between transcription and translation were achieved in the engineered mutant, which traffics to the ER and then to lipid droplets as cells reach stationary phase. In comparison to the wild type, lipid droplet targeting increases expression levels by more than 20-fold. Our second approach builds on the concept of enzyme co-localization. The short chain alcohol-O-acetyltransferase Atf1 natively localizes to the surface of lipid droplets, which is necessary for high activity. A synthetic protein scaffold based on the plant protein oleosin was engineered to co-localize upstream enzymes of the ester biosynthesis pathway to the surface of lipid droplets. Optimization of the scaffold architecture and enzyme expression levels enhanced ester biosynthesis by nearly 2-fold. Combined, these two examples demonstrate new post translation strategies with the potential to provide subcellular engineering solutions to other membrane-bound pathways.